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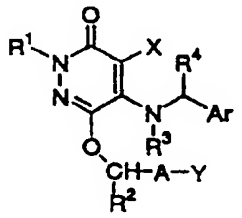
权利要求书6页 说明书24页 附图页数2页

[54]发明名称 勃起功能障碍治疗剂

[57]摘要

本发明提供一种勃起功能障碍的治疗剂,该治疗剂包括以式(Ⅰ)表示的3(2H)-哒嗪酮衍生物或其药用盐作为活性成分,其中的各个符号与说明书中的定义相同。



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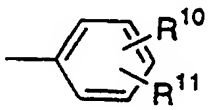
$C_{1-4}$烷基或卤原子取代的苯基， 或表示 $S(O)_m-R^{12}$ ，其中 m 是 0-2 的整数和 $R^{12}$ 表示 $C_{1-4}$ 烷基。

5. 权利要求1-3任意一项所述的勃起功能障碍治疗剂，其中Ar是吡啶基、N-氧化吡啶基或被$OR^{22}$取代的苯基，其中$R^{22}$表示$C_{1-4}$烷基。

6. 权利要求1-5任意一项所述的勃起功能障碍治疗剂，其中A是直链或支链的$C_{1-8}$亚烷基，其中直链上的碳原子可被一个羟基（-OH）取代。

7. 权利要求4-6任意一项所述的勃起功能障碍治疗剂，其中Y是下式表示的苯基：




其中 $R^{10}$ 和 $R^{11}$ 各自独立地表示氢原子，卤原子，$C_{1-4}$ 烷基，$C_{1-4}$ 酰氨基，$OR^5$，其中 $R^5$表示氢原子或 $C_{1-4}$烷基，$NHSO_2R^9$，其中 $R^9$表示被氢原子、$C_{1-4}$ 烷基或卤原子取代的苯基， 或表示 $S(O)_m-R^{12}$ ，其中 m 是 0-2 的整数和 $R^{12}$ 表示 $C_{1-4}$ 烷基。

8. 权利要求7所述的勃起功能障碍治疗剂，其中Y是被卤原子取代的苯基。

9. 权利要求5-8任意一项所述的勃起功能障碍治疗剂，其中Ar是3-吡啶基基团。

10. 权利要求1所述的勃起功能障碍治疗剂，其中式（I）表示的化合物是4-溴-6-[3-(4-氯苯基)丙氧基]-5-(3-吡啶基甲基氨基)-3-(2H)-哒嗪酮或4-溴-6-[3-(4-氯苯基)-3-羟基-丙氧基]-5-(3-吡啶基甲基氨基)-3-(2H)-哒嗪酮，或其药用盐。

温和的。

本发明的哒嗪酮化合物(I)具有 PDE III 和 PDE V 两种抑制活性，可用作勃起功能障碍的治疗剂，与本领域的治疗方法相比其应用得到改进。

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# Inhibition of allergen-induced lung eosinophilia by type-III and combined type III- and IV-selective phosphodiesterase inhibitors in Brown-Norway rats

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**Abstract.** We examined the effect of a type IV (rolipram) and a combined type III and IV (Org 20421) isoenzyme-selective phosphodiesterase inhibitor upon allergen-induced pulmonary eosinophil recruitment in sensitised Brown Norway rats. Rats were sensitised with ovalbumin intraperitoneally and later challenged with ovalbumin aerosol which induced a significant increase in the total eosinophil and neutrophil count in bronchovalveolar lavage fluid at 24 hours (from $0.38 \pm 0.12$ to $1.36 \pm 0.18 \times 10^6$, $p < 0.01$ and from $0.06 \pm 0.01$ to $0.33 \pm 0.07 \times 10^6$, $p < 0.01$) respectively. Pretreatment with rolipram (30 μmol/kg) and Org 20421 (30 μmol/kg) abolished the eosinophilia and neutrophilia evoked by ovalbumin. We conclude that type IV and possibly type III isozyme phosphodiesterase inhibitors may regulate, directly or indirectly, eosinophil and neutrophil activity and/or those cells responsible for attracting them into the lung.

**Key words:** Org 20241 – Rolipram – Eosinophilia – Asthma – Brown-Norway rat

## Introduction

Pulmonary eosinophilia is a feature of asthma, and eosinophils have been implicated in the pathogenesis of epithelial cell damage and bronchial hyperresponsiveness observed in asthma [1]. The mechanisms underlying the recruitment of eosinophils to the lungs are unclear but recent evidence suggests that this may be partly dependent on activated CD4+ T-lymphocytes of the Th-2 subset and the subsequent release of cytokines including IL-3, IL-5 and GMCSF [2].

Cyclic nucleotides regulate the activity of many cells in the airways, including proinflammatory immunocompetent cells such as macrophages, eosinophils, mast cells and lymphocytes [3]. Cyclic purine phosphodiesterases (PDE) are responsible for the inactivation of cyclic 3′,5′-adenosine monophosphate (cAMP) and cyclic 3′,5′-guanosine monophosphate (cGMP). At least seven PDE isoenzyme families have now been characterised [4, 5] which hydrolyse cAMP and/or cGMP, representatives of which are differentially expressed between cells and tissues. In inflammatory cells, the predominant PDE's expressed are the types III and IV isoenzymes [6]. *In-vitro* studies have established that inhibition of these isoenzymes by selective PDE inhibitors suppresses various aspects of cell activation [3, 7–9].

To determine whether selective PDE inhibitors possess inhibitory activity *in vivo*, we examined the ability of a type IV PDE isoenzyme inhibitor, rolipram [10] and of a combined Type III and IV isoenzyme inhibitor Org 20241 [11] to inhibit allergen-induced eosinophil accumulation into the lungs of ovalbumin-sensitised Brown Norway rats. This species was chosen because it mimics several of the features characteristic of asthma, including the recruitment of eosinophils to the lungs following allergen challenge [12].

## Materials and methods

### *Sensitisation procedure and allergen exposure*

Inbred female Brown Norway rats (weight: 180–200 grams) were sensitised by intraperitoneal (ip) injection of 1 ml of a suspension of 1 mg ovalbumin (OA) and 100 mg of aluminium hydroxide ($Al(OH)_3$) in 0.9% (wt/vol) saline given on three successive days. Seventeen to twenty-one days after the initial intraperitoneal injection, rats were exposed to an aerosol of either 1% OA or saline and 18–24 hours later, they were anaesthetised and bronchoalveolar lavage fluid collected.

### *Protocol*

Four groups of sensitised rats were used:

*Group A* (control – saline aerosol n = 7). 17–21 days after sensitisation, rats were injected i.p. with the first of three placebo

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(DMSO) injections given at 24 h, 6 h and 0.5 h prior to a single saline aerosol exposure for 15 min. Rats were studied 18–24 hours later.

*Group B* (placebo – allergen aerosol, n = 8). Same as Group B above, except that the rats were exposed to a 1% ovalbumin aerosol for 15 min.

*Group C* (rolipram – allergen aerosol, n = 8). 17–21 days after sensitisation, rats were injected i.p. with rolipram (30 μmol/kg) administered at 24 h, 6 h and 0.5 h prior to single allergen aerosol exposure for 15 min. Rats were studied 18–24 h later. This dose of rolipram has previously been shown to prevent antigen- and mediator-induced bronchoconstriction [13, 14].

*Group D* (Org 20241 – allergen aerosol, n = 8). Same as Group C, except that Org 20241 (30 μmol/kg) was administered instead.

### *Aerosol exposure*

Aerosol exposure was accomplished by placing the rats in a 6.5 litre plexiglass chamber. This was connected to a Devilbiss Pulmosonic nebuliser (Model No 2512, Feltham, Middlesex, UK) which generated an aerosol mist which was pumped into the exposure chamber by the airflow supplied from a small animal ventilator set at 10 strokes $min^{-1}$ with a pumping volume of 10 ml.

### *Bronchoalveolar lavage*

Rats were given an overdose of sodium pentobarbitone (100 mg/kg iv) and the lungs were lavaged with 10 × 2 ml aliquots of 0.9% w/v sterile saline via a polyethylene tube through an upper tracheostomy site. Lavage fluid was centrifuged (× 500 g for 10 min at 4 °C) and the cell pellet was resuspended in 0.5 ml of Hank's balanced salt solution. Total cell counts were made by adding 10 μl of the cell suspension to 90 μl of Kimura stain and counted in a Neubauer chamber under a light microscope. Differential cell counts were made from cytospin preparations stained with May-Grunwald stain. Cells were identified as eosinophils, lymphocytes, neutrophils and macrophages by standard morphology and 500 cells were counted under × 400 magnification. The percentage and absolute number of each cell type were calculated.

### *Data analysis*

Data are reported as mean ± SEM. Non-parametric analysis of variance (Kruskal-Wallis method) was used to determine significant variance among the groups. If a significant variance was found, Mann-Whitney U-test was used to analyse for significant differences between individual groups. $p < 0.05$ was considered to be significant.

## Results

### *Aerosol exposure*

Rats sensitised by i.p. injection of OA responded when exposed to 1% OA aerosol solution by showing obvious respiratory distress characterised by the development of a defensive posture and exaggerated laboured breathing movements. This immediate reponse started within 2–4 mins and all animals recovered spontaneously. This response was not inhibited by pretreatment of the rats with either rolipram or Org 20241.

### *Cellular content of BAL fluid*

There was a significant increase in total numbers of eosinophils and neutrophils in BAL fluid after placebo pretreatment (Group 2) when compared to control rats (Group 1), from $0.38 \pm 0.12$ and $0.06 \pm 0.01$ to $1.36 \pm 0.18$ and $0.33 \pm 0.07 \times 10^6$, respectively. There was no significant change in macrophage or lymphocyte count. The increase in the number of neutrophils and eosinophils was significantly inhibited by pretreatment of rats with both rolipram and Org 20241 to levels similar to those observed in control animals (Group A) (Fig. 1). The eosinophil count after Org 20421 and rolipram was $0.28 \pm 0.11$ and $0.27 \pm 0.13 \times 10^6$ respectively ($P < 0.001$ compared to placebo treatment). Org 20421 and rolipram reduced neutrophil numbers to $0.12 \pm 0.03$ and $0.03 \pm 0.01 \times 10^6$ cells respectively ($P < 0.001$) (Fig. 1).

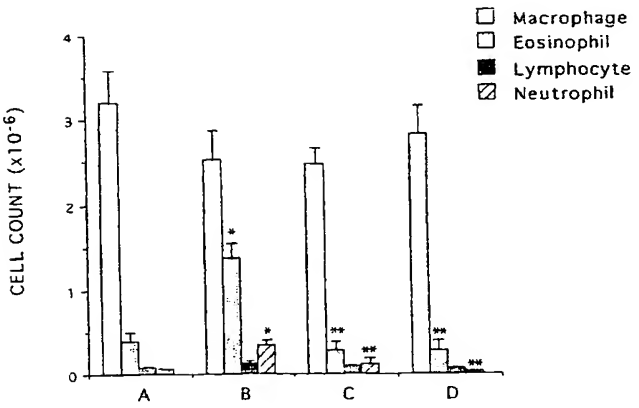


**Fig. 1.** Total macrophage, eosinophil, lymphocyte and neutrophil counts (from left to right) in sensitised Brown-Norway rats exposed to saline (group A), exposed to ovalbumin (OA) (group B) and exposed to OA but pretreated with rolipram (group D) or Org 20241 (group C). Data presented as mean ± SEM. *$p < 0.01$ compared to group A and **$p < 0.001$ when compared to group B.

## Discussion

We have shown that rolipram, a type IV PDE inhibitor, and Org 20241, a mixed inhibitor of the PDE III and PDE IV isozyme families, suppressed the infiltration of eosinophils into bronchoalveolar lavage fluid of sensitised Brown-Norway rats in response to ovalbumin provocation. In addition, the influx of neutrophils was also inhibited. Our data thus support a potential anti-inflammatory role for PDE IV inhibitors in this species; a role for PDE III isozyme cannot be excluded. Similar data have been reported for rolipram [14], zardaverine [15] and benzafentrine [16, 17] in the guinea-pig. The importance of our findings is that inhibition of eosinophilia and neutrophilia is demonstrated in an animal model of asthma that mimics closely in many respects asthma in man. Unlike the guinea-pig, Brown-Norway rats are IgE-producing animals and corticosteroid-sensitive [18, 19].

The mechanisms underlying the accumulation of eosinophils in the airways following allergen challenge are unclear. This accumulation may depend upon activation of T-lymphocytes to release factors that may

induce the recruitment and prolonged survival of eosinophils, such as the cytokines interleukin (IL)-3, IL-5 and GM-CSF [2]. We have previously shown that there is an increase in $CD25^+$ lymphocytes in bronchovalveolar lavage fluid in sensitised Brown-Norway rats challenged with ovalbumin [20]. In previous studies, glucocorticosteroids have also been shown to inhibit eosinophil accumulation in this model [18]; in addition, a more specific inhibitor of activated T-lymphocytes, cyclosporin, was effective in inhibiting eosinophil accumulation [18]. Although it is possible that rolipram and Org 20241 prevent the release of chemoattractants from T-cells, they may also be working through other mechanisms, such as inhibition of macrophage activation [21] or eosinophil locomotion [22]. There is also evidence for an effect of PDE IV inhibitors at the level of the pulmonary microvenular endothelium [23, 24].

PDE IV is a major cAMP-hydrolysing isoenzyme in all pro-inflammatory and immunocompetent cells such as T-lymphocytes [25], monocytes [26], neutrophils [27] and eosinophils [7, 28]. The T-lymphocyte also contains a significant amount of PDE III activity [8, 25]. PDE III or PDE IV inhibitors attenuate thymidine incorporation induced by phytohemagglutinin in T-lymphocytes, with a much greater inhibition achieved when they are used in combination [25]. More recent studies have shown that PDE IV inhibitors suppress IL-2, IL-4 and INF$\gamma$ released from T-cells [29, 30] by a mechanism that may involve reduced gene transcription [31]. These effects were similarly enhanced by a PDE III inhibitor. These observations are in line with our *in-vivo* studies, although we have not shown at the doses we have chosen any greater effectiveness of the combined type III and type IV inhibitor, Org 20421. It is likely that we were using maximally effective doses.

We found that both rolipram and Org 20421 did not inhibit OA-induced acute signs of respiratory distress following allergen exposure. We did not measure lung function at this time-point and our study was not designed to examining the acute bronchoconstrictor response. However, there has been reports of rolipram inhibiting ovalbumin-induced bronchoconstriction in sensitised guinea-pigs [14]. Because rolipram minimally affected exogenous spasmogen-induced airway constriction [14], it has been proposed that rolipram may inhibit mast cell degranulation.

In summary, we have shown that inhibition of type IV and possibly type III PDE isoenzymes prevents eosinophil and neutrophil accumulation into the lungs of sensitised Brown-Norway rats induced by allergen challenge. These observations suggest that these inhibitors possess anti-inflammatory properties.

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## References

[1] Laitinen LA, Heino M, Laitinen A. Airway hyperresponsiveness, epithelial disruption and epithelial inflammation. In: Farmer SF, Hay DWP, eds. The Airway Epithelium: physiology, pathophysiology and pharmacology. New York: Dekker, 1990:187–211.

[2] Corrigan CJ, Kay AB. Cells and eosinophils in the pathogenesis of asthma. Immunol Today 1992;13:501–7.

[3] Torphy TJ, Undem BD. Phosphodiesterase inhibitors: new opportunities for the treatment of asthma. Thorax 1992; 416:512–23.

[4] Beavo JA, Reifsnyder DH. Primary sequence of cyclic nucleotide phosphodiesterase isozymes and the design of selective inhibitors. Trends Pharmacol Sci 1990;11:150–5.

[5] Michaeli T, Bloom TJ, Martins T, et al. Isolation and characterization of a previously undetected human cAMP phosphodiesterase by complementation of cAMMP phosphodiesterase-deficient *saccharomyces cerevisiae*. J Biol Chem 1993;268:12925–32.

[6] Nicholson CD, Shahid M. Inhibitors of cyclic nucleotide phosphodiesterase isoenzymes – their potential utility in the therapy of asthma. Pulmonary Pharmacol 1994;7:1–17.

[7] Dent G, Giembycz MA, Rabe KF, Barnes PJ. Inhibition of eosinophil cyclic nucleotide PDE activity and opsonized zymosan-stimulated respiratory burst by "Type IV"-selective PDE inhibitors. Br J Pharmacol 1991;103:1339–46.

[8] Robicsek SA, Krzanowski JJ, Szentiranyi A, Polson JB. High pressure liquid chromatography of cyclic nucleotide phosphodiesterase from purified human T-lymphocytes. Biochem Biophys Res Commun 1989;163:554–60.

[9] Giembycz MA. Could isoenzyme-selective phosphodiesterase inhibitors render bronchodilator therapy redundant in the treatment of bronchial asthma. Biochem Pharmacol 1993;43: 2041–51.

[10] Reeves ML, Leigh BK, Rink TJ. The identification of a new cyclic nucleotide phosphodiesterase in human and guinea-pig cardiac ventricle. Biochem J 1987;241:535–41.

[11] Nicholson CD, Shahid M, Bruin J, et al. Org 20241, a cyclic nucleotide phosphodiesterase inhibitor for asthma. Br J Pharmacol 1992;107:252.

[12] Elwood W, Lotvall JO, Barnes PJ, Chung KF. Characterisation of allergen-induced inflammation and bronchial hyperresponsiveness in sensitised Brown-Norway rats. J Allergy Clin Immunol 1991;88:951–60.

[13] Howell RE, Sickels BD, Woeppel SL. Pulmonary antiallergic and bronchodilator effects of isozyme-selective phosphodiesterase inhibitors. J Pharmacol Exp Ther 1993; 246:609–15.

[14] Underwood DC, Osborn RR, Novak LB, et al. Inhibition of antigen-induced bronchoconstriction and eosinophil infiltration in the guinea-pig by the cyclic AMP-specific phosphodiesterase inhibitor, rolipram. J Pharmacol Exp Ther 1993;266: 306–10.

[15] Schudt C, Winder S, Litze M, Kilian U, Beume R. Zardaverine: a cyclic AMP specific PDE III/IV inhibitor. Agents Actions 1991;34:161–77.

[16] Sanjar S, Aoki S, Kristersson A, Smith D, Morley J. Antigen challenge induces pulmonary airway eosinophil accumulation and airway hyperreactivity in sensitised guinea-pigs: the effect of anti-asthma drugs. Br J Pharmacol 1990;99: 679–86.

[17] Kings MA, Chapman I, Kristersson A, Sanjar S, Morley J. Human recombinant lymphokines and cytokines induce pulmonary eosinophilia in the guinea-pig which is inhibited by ketotifen and AH 21-132. Int Arch Allergy Appl Immunol 1990;91:354–61.

[18] Elwood W, Lotvall J, Barnes PJ, Chung KF. Effects of cyclosporin A and dexamethasone on allergen-induced bronchial hyperresponsiveness and inflammatory cell. Am Rev Respir Dis 1992;145:1289–94.

[19] Waserman S, Olivenstein R, Renzi P, Xu L-J, Martin JG. The relationship between late asthmatic responses and antigen-specific immunoglobulin. J Allergy Clin Immunol 1992;90: 661–9.

[20] Haczku A, Chung KF, Sun J, Barnes PJ, Kay AB, Moqbel R. Lymphocyte populations in peripheral blood and

bronchoalveolar lavage of Brown-Norway rats sensitized and exposed to ovalbumin. Am Rev Resp Dis 1993;147:A853.
[21] Schudt CHR, Tenor H, Wendel A, et al. Effect of selective phosphodiesterase (PDE) inhibitors on activation of macrophages and lymphocytes. Naunyn Schmiedebergs Arch Pharmacol 1992;345:92.
[22] Tanimoto Y, Maruo H, Bewtra AK, Townley RG. Effects of phosphodiesterase IV inhibitor (WAY-PDE-641) on human eosinophil and neutrophil migration *in vitro*. J Allergy Clin Immunol 1994;93:257 (abstr).
[23] Raeburn D, Karlsson JA. Effects of isoenzyme-selective inhibitors of cyclic nucleotide phosphodiesterase on microvascular leak in guinea-pig airways. J Pharmacol Exp Ther 1993;267:1147–52.
[24] Ortiz JL, Cortijo J, Valles JM, Bou J, Morcillo EJ. Rolipram inhibits PAF-induced airway microvascular leakage in guinea-pig: a comparison with milrinone and theophylline. Fund Clin Pharmacol 1992;6:247–9.
[25] Robicsek SA, Blanchard DK, Djeu JY, Krzanowski JJ, Stertivanyi A, Polson JB. Multiple high-affinity cAMP-phosphodiesterases in human T-lymphocytes. Biochem Pharmacol 1991;42:869–77.
[26] Godfrey RW, Manzi RM, Genarro DE, Hofstein ST. Phospholipid and arachidonic acid metabolism in zymosan-stimulated human monocytes: modulation by cAMP. J Cell Physiol 1987;131:384–92.
[27] Nielson CP, Vestal RE, Sturm RJ, Heaslip R. Effects of selective phosphodiesterase inhibitors on the polymorphonuclear leukocyte respiratory burst. J Allergy Clin Immunol 1990;86:801–8.
[28] Souness JE, Carter CM, Diocee BK, Hassall GA, Wood LJ, Turner NC. Characterization of guinea-pig eosinophil phosphodiesterase activity. Assessment of its involvement in regulating superoxide generation. Biochem Pharmacol 1991; 42:937–45.
[29] Giembycz MA, Corrigan CJ, Kay AB, Barnes PJ. Inhibition of CD4 and CD8 T-lymphocytes (T-LC) proliferation and cytokine secretion by isoenzyme-selective phosphodiesterase (PDE) inhibitors: correlation with intracellular cyclic AMP (cAMP) concentrations. J Allergy Clin Immunol 1994;93:167 (abstr).
[30] Crocker IC, Townley RG, Khan MM. Phosphodiesterase inhibitors modulate cytokine secretion and proliferation. J Allergy Clin Immunol 1994;93:286 (abstr).
[31] Essayan DM, Kagey-Sobotka A, Lichtenstein LM, Huang S-K. Studies of phosphodiesterase (PDE) isozyme function and expression in human allergic disease. J Allergy Clin Immunol 1994;93:286 (abstr).